The U_S3 protein kinase of herpes simplex virus 1 mediates the posttranslational modification of BAD and prevents BAD-induced programmed cell death in the absence of other viral proteins

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Earlier studies have shown that the d120 mutant of herpes simplex virus 1, which lacks both copies of the α4 gene, induces apoptosis in all cell lines tested. In some cell lines d120-induced apoptosis, manifested by the release of cytochrome *c*, activation of caspase 3, and fragmentation of cellular DNA, is blocked by the overexpression of Bcl-2. In these cells viral protein kinase U_S3 delivered in *trans* blocks apoptosis induced by the mutant virus at a premitochondrial stage. We report that the U_S3 protein kinase targets the pro-apoptotic BAD member of the Bcl-2 family. Specifically, the U_S3 protein kinase mediates a posttranslational modification of BAD and blocks its cleavage, which is reported to activate apoptosis. Thus, U_S3 protein kinase is the sole viral protein required to block activation of caspase 3, prevent cleavage of poly(ADP-ribose) polymerase, and block fragmentation of cellular DNA induced by BAD.

In earlier publications this and other laboratories reported that the interaction of herpes simplex virus 1 (HSV-1) with cells results in programmed cell death and that the virus has evolved mechanisms that block apoptosis, whether it is induced by viral gene products or by exogenous agents. Specifically, wild-type HSV does not induce apoptosis, and infection with wild-type virus blocks apoptosis induced by osmotic or thermal shock or by Fas ligand (1–7). A number of HSV-1 mutants have been reported to induce apoptosis. These include mutants lacking the infected cell protein no.4 or infected cell protein no. 27 (4, 8, 9), two regulatory proteins expressed immediately after infection, a mutant lacking glycoprotein D (10), and a mutant carrying a temperature-sensitive mutation that blocks the release of viral DNA from capsids at nuclear pores in cells infected and maintained at nonpermissive temperatures (3, 11). Detailed analyses of the mutant d120 from which both copies of the gene encoding infected cell protein no. 4 had been deleted revealed that the virus induces apoptosis in all of the cell lines tested but that the mechanisms by which the virus induces apoptosis is cell type dependent (5). In HEp-2 cells the d120 mutant caused the translocation of cytochrome *c* from mitochondria, activation of caspase 3, and fragmentation of cellular DNA (5, 12). Apoptosis was blocked in a HEp-2-derived cell line that overexpressed Bcl-2 (12).

Earlier studies have also reported that d120 rescuants in which the deleted gene encoding infected cell protein no. 4 was repaired continued to induce apoptosis but that DNA fragments sharing the U_S3 gene blocked apoptosis (13). Other laboratories have since confirmed that the U_S3 protein kinase contributes to HSV-mediated protection from a variety of exogenous apoptotic inducers (7, 14–16). In a recent study we have shown that the U_S3 protein kinase blocked d120-induced apoptosis at a premitochondrial stage and that activation of caspase 3 could be blocked by the U_S3 protein kinase expressed as late as 6 to 9 h after infection of HEp-2 cells with the d120 mutant (17). Because overexpression of U_S3 blocked d120-induced apoptosis at a premitochondrial stage, these studies suggested the possibility that the U_S3 protein kinase targets a member of the Bcl-2 protein family.

Members of the Bcl-2 family of proteins regulate the execution of programmed cell death. The members of this family can be functionally separated into apoptotic antagonists, including Bcl-2, Bcl-X_L, and Bcl-w, and apoptotic agonists, such as BAD, BID, and BAX. These key apoptotic regulators mediate their pro- or antiapoptotic signals through their relative abundance, subcellular localization, and posttranslational modifications.

Pro- and antiapoptotic family members are capable of dimerizing through the three Bcl-2 homology domains (BH1, BH2, and BH3), apparently titering out each other's functions (18–20). Specifically, BH1, BH2, and BH3 domains form a hydrophobic cleft to which the BH3 domain can bind (21). Some proapoptotic Bcl-2 family members, such as BAD, contain only the BH3 domain, which is essential for binding to antiapoptotic family members, such as Bcl-2 and Bcl-X_L, and for their proapoptotic function (19).

Cell survival signals block BAD from inducing apoptosis by phosphorylation (22). Some of these signals activate phosphatidylinositol 3-kinase with subsequent activation of Akt, which phosphorylates BAD at Ser-136 (23, 24). Survival signals also promote the activation of 90-kDA ribosomal S6 kinase (RSK) and protein kinase A (PKA), which have both been shown to phosphorylate BAD at Ser-112 (25, 26). Phosphorylation of BAD at Ser-112 and Ser-136 has been demonstrated to abrogate its proapoptotic activity by promoting its association with 14-3-3 proteins, which sequester phosphorylated BAD, thereby preventing its localization to the mitochondria and association with Bcl-X_L (26–29). Furthermore, phosphorylation of BAD at Ser-155 disrupts its interaction with Bcl-X_L (30–32). Conversely, activation of BAD appears to be carried out by phosphatases. Thus two different phosphatases, calcineurin and PP1α, dephosphorylate BAD at Ser-112 and Ser-136, thereby releasing BAD from 14-3-3 proteins, stimulating its binding to Bcl-2/Bcl-X_L and ultimately leading to cytochrome *c* release, caspase activation, and apoptosis (33, 34). Recently it has been reported that death receptor engagement induces the caspase-mediated cleavage of BAD, yielding an M_r 15,000 truncated protein that is a more potent inducer of apoptosis than the full-length BAD (35). This cleavage could serve as a general mechanism to maintain and enhance the proapoptotic functions of BAD.

In this report, we show that the U_S3 protein kinase, in the absence of other HSV proteins, posttranslationally modified the

Abbreviations: HSV, herpes simplex virus; PARP, poly(ADP-ribose) polymerase; Bac, baculovirus; pfu, plaque-forming units.

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